

Prediction of portal and hepatic blood flow from intake level data in cattle

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1 **INTERPRETIVE SUMMARY:**

2 **Prediction of portal and hepatic blood flow in cattle.** Ellis et al. Given the extent of variability in
3 post absorptive metabolism, there is growing interest in developing integrated post-absorptive
4 metabolism models for cattle. An integral part of linking a multi-organ post-absorptive model is
5 the prediction of nutrient flow between organs, and thus blood flow. This paper applied a
6 multivariate meta-analysis technique to simultaneously predict incoming and outgoing blood flows
7 to the liver. Prediction equations based on DMI performed well, and division of DMI into forage
8 and concentrate DMI improved blood flow predictions.

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10 **RUNNING HEAD: PREDICTION OF LIVER BLOOD FLOW IN CATTLE**

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12 **Prediction of portal and hepatic blood flow from intake level data in cattle**

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ABSTRACT

There is growing interest in developing integrated post-absorptive metabolism models for dairy cattle. An integral part of linking a multi-organ post-absorptive model is the prediction of nutrient fluxes between organs, and thus blood flow. It was the purpose of this paper to use a multivariate meta-analysis approach to model portal blood flow (PORBF) and hepatic venous blood flow (HEPBF) simultaneously, with evaluation of hepatic arterial blood flow (ARTBF; $\text{ARTBF} = \text{HEPBF} - \text{PORBF}$) and $\text{PORBF}/\text{HEPBF}$ (%) as calculated values. The database used to develop equations consisted of 296 individual animal observations (lactating and dry dairy cows and beef cattle) and 55 treatments from 17 studies, and a separate evaluation database consisted of 34 treatment means (lactating dairy cows and beef cattle) from 9 studies obtained from the literature. Both databases had information on DMI, MEI, body weight and a basic description of the diet including crude protein intake and forage proportion of the diet (FP; %). Blood flow (L/h or L/kg $\text{BW}^{0.75}/\text{h}$) and either DMI or MEI (g or MJ/d or g or MJ/kg $\text{BW}^{0.75}/\text{d}$) with linear and quadratic fits were examined. Equations were developed using cow within experiment and experiment as random effects, and blood flow location as a repeated effect. Upon evaluation with the evaluation database, equations based on DMI typically resulted in lower root mean square prediction errors, expressed as a % of the observed mean (rMSPE%) and higher concordance correlation coefficient (CCC) values than equations based on MEI. Quadratic equation terms were frequently non-significant, and the quadratic equations did not out-perform their linear counterparts. The best performing blood flow equations were: $\text{PORBF (L/h)} = 202 (\pm 45.6) + 83.6 (\pm 3.11) \times \text{DMI (kg/d)}$ and $\text{HEPBF (L/h)} = 186 (\pm 45.4) + 103.8 (\pm 3.10) \times \text{DMI (kg/d)}$, with rMSPE% values of 17.5 and 16.6 and CCC values of 0.93 and 0.94, respectively. The residuals (predicted – observed) for $\text{PORBF}/\text{HEPBF}$ were significantly related to the forage % of the diet, and thus equations for

PORBF and HEPBF based on forage and concentrate DMI were developed: $\text{PORBF (L/h)} = 210 (\pm 51.0) + 82.9 (\pm 6.43) \times \text{Forage (kg DM/d)} + 82.9 (\pm 6.04) \times \text{Concentrate (kg DM/d)}$, and $\text{HEPBF (L/h)} = 184 (\pm 50.6) + 92.6 (\pm 6.28) \times \text{Forage (kg DM/d)} + 114.2 (\pm 5.88) \times \text{Concentrate (kg DM/d)}$, where rMSPE% values were 17.5 and 17.6 and CCC values were 0.93 and 0.94, respectively. Division of DMI into forage and concentrate fractions improved the joint Bayesian Information Criterion (BIC) value for PORBF and HEPBF (BIC = 6512 vs. 7303), as well as slightly improved the rMSPE and CCC for ARTBF and PORBF/HEPBF. This was despite minimal changes in PORBF and HEPBF predictions. Developed equations predicted blood flow well, and could easily be used within a post absorptive model of nutrient metabolism. Results also suggest different sensitivity of PORBF and HEPBF to the composition of DMI, and accounting for this difference resulted in improved ARTBF predictions.

Key words: blood flow, portal, hepatic, cattle, meta-analysis, multivariate

INTRODUCTION

The ability of current feed ration systems to predict the effects of metabolizable protein supply on milk protein production and nitrogen excretion to the environment by dairy cattle is limited by an oversimplified representation of post-absorptive metabolism (Lapierre et al., 2006). Given the variability in post-absorptive metabolism, there is interest in developing integrated post-absorptive models of metabolism (portal-drained viscera, liver, mammary gland, and other organs or tissues) to replace current empirical feeding systems for cattle. Integration of such organ-based models requires prediction of nutrient flow between organs, including prediction of hepatic arterial (**ARTBF**), portal (**PORBF**) and hepatic venous (**HEPBF**) blood flows (**BF**). Across the liver, the

relative contribution of ARTBF and PORBF can have a significant effect on nutrient fluxes through the organ (e.g. Barnes et al., 1986), warranting reliable prediction of these blood flows. Nutrient concentration in PORBF is modified by the net absorption of nutrients following digestion of feeds (or the net utilization of nutrients from arterial blood), while ARTBF nutrient concentration is mainly the result of the residual balance between nutrient absorption, utilization, endogenous synthesis, and mobilization from body tissues. Several attempts to model ARTBF, PORBF and/or HEPBF in ruminants are present in the literature, but 1.) were conducted on sheep (e.g. Vernet et al., 2009), 2.) use older meta-analysis techniques which exclude random effects (e.g. Lescoat et al., 1996), or 3.) examined only one of the 3 blood flows of interest (e.g. Huntington, 1984; Bermingham et al., 2008). Species differences in blood flow (e.g. between cattle and sheep) have already been observed (Vernet et al., 2005; Bermingham et al., 2008), indicating that cross-species application of blood flow equations may be poor. Equations developed using older meta-analysis techniques may inherently contain prediction errors (St-Pierre, 2001; Sauvant et al., 2008). A fully integrated post-absorptive model for cattle would require all 3 blood flows to be estimated simultaneously. Therefore, a multivariate meta-analysis approach, simultaneously fitting equations for ARTBF, PORBF and HEPBF, while accounting for the interrelationship between BF, is warranted.

The purpose of this study was therefore to (1) investigate the simultaneous prediction of ARTBF, HEPBF and PORBF for cattle via a multivariate meta-analysis on published studies, considering DMI and MEI as driving variables, and (2) to compare these predictions to available extant prediction equations on an evaluation database, in order to identify the most appropriate prediction equations for use in future cattle metabolism models.

MATERIALS AND METHODS

Developmental Database

The database used for equation development is summarized in Table 1. It consisted of 17 studies with 296 individual animal means and 55 treatment means. Published experiments included: Reynolds et al. (1991; 1992a,b; 1993; 1994a,b; 1995a,b; 1998; 1999; 2001; 2003a,b), Caton et al. (2001), Hanigan et al. (2004), Maltby et al. (2005) and Røjen et al. (2011). Experiments covered both lactating and dry dairy cows and growing beef cattle (steers and heifers). Method of BF measurement was downstream dilution of para-aminohippuric acid (PAH) (Katz and Bergman, 1969) for all studies. Within studies, BF results were means of (between) 5 to 12 hourly measurements. All reported BF values are on a whole blood basis. Criteria for inclusion in the developmental database included availability of individual animal data and provision of information on both PORBF and HEPBF, DMI, metabolizable energy intake (**MEI**), BW and forage % (**FP**) in the diet. Within study, any treatments which were not nutritional were removed in order to minimize non-nutritional variation in the database.

Within the database, the average SD within treatment across the database (indicator of within treatment animal variability) was 135 L/h, 210 L/h, 177 L/h and 0.852 kg/d for ARTBF, PORBF, HEPBF and DMI, respectively, and the average SD of treatment means (indicator of variation across treatment means) was 152 L/h, 548 L/h, 673 L/h and 6.35 kg/d for ARTBF, PORBF, HEPBF and DMI, respectively. Preliminary analysis (not shown) revealed that within-treatment BF variation was significantly related to within-treatment DMI variation ($P < 0.01$).

Evaluation Database

The database used for equation evaluation is summarized in Table 2. It consisted of 9

studies with 34 treatment means extracted from the published literature (Wieghart et al., 1986; Eiseman and Nienaber, 1990; Huntington et al., 1990; Guerino et al., 1991; Reynolds and Tyrrell, 1991; Casse et al., 1994; Eiseman and Huntington, 1994; Whitt et al., 1996; Alio et al., 2000), and included both lactating dairy cows and beef cattle. Method of BF measurement for all studies was downstream dilution of PAH (Katz and Bergman, 1969). Similar to the developmental database, all reported BF values are whole blood. Criteria for inclusion in the database included published studies with provision of information on PORBF, HEPBF, DMI, MEI, BW and FP. Having MEI and simultaneous reporting of PORBF and HEPBF as inclusion criteria for the evaluation database limited the number of potential studies which could be included, but ensured an equal comparison between DMI and MEI, and PORBF and HEPBF based equations. Similar to the developmental database, within study, any treatments which were not nutritional were removed in order to minimize non-nutritional variation in the database.

The observed PORBF and HEPBF vs. DMI relationship for both the developmental and evaluation databases are presented in Figure 1 and the distribution of FP across DMI in Figure 2.

Equation Development

To model the effect of DMI and MEI of cattle on ARTBF, PORBF and HEPBF, mixed model analysis was performed. Linear and quadratic multivariate mixed model analysis was conducted using the NLINMIX macro of SAS (NLMM 8.0 SAS; Moser, 2004; Littell et al., 2006), with simultaneous parameterization of the response variables (PORBF, HEPBF) and representation of the correlation between these variables via the repeated effects statement (Strathe et al., 2010). For a recent example of NLMM code, see the appendix of Strathe et al. (2009).

Due to the high degree of error and low sensitivity of ARTBF to the driving variables, it

was difficult to obtain convergence of the multivariate model when ARTBF was modelled directly (not shown). This is likely because ARTBF is a comparatively small flow determined by difference experimentally (*in vivo*, observed ARTBF = observed HEPBF – observed PORBF). As a result, predicted ARTBF was determined by calculation of the difference between predicted PORBF and HEPBF. Similarly, PORBF/HEPBF (%) was evaluated as the ratio of predicted blood flows, and not modeled directly.

As the data were compiled from multiple studies, it was necessary to analyze not only the fixed effects of the dependent variables, but also the random effect of experiment as this accounts for differences between experiments such as physiological status of the animals, experimental design, measurement methods, techniques, and laboratory variation (St-Pierre, 2001; Sauvant et al, 2008). As it was desirable to examine the between animal variation in DMI and BW, the full model also included the random effect of cow nested within experiment.

The statistical model can be written as follows, where fixed and random effects are incorporated directly into parameters:

$$Y_{ijk} = f(\emptyset_{ij}, \text{intake}_{ijk}) + e_{ijk}, \quad [1]$$

$$\emptyset_{ij} = \begin{bmatrix} \emptyset_{1ij} \\ \emptyset_{2ij} \\ \vdots \\ \emptyset_{dij} \end{bmatrix} = \begin{bmatrix} B_{11} \cdot x_1 + B_{12} \cdot x_2 \\ B_{21} \cdot x_1 + B_{22} \cdot x_2 \\ \vdots \\ B_{d1} \cdot x_1 + B_{d2} \cdot x_2 \end{bmatrix} + \begin{bmatrix} b_i^{(1)} \\ b_i^{(2)} \\ \vdots \\ b_i^{(d)} \end{bmatrix} + \begin{bmatrix} b_{i,j}^{(1)} \\ b_{i,j}^{(2)} \\ \vdots \\ b_{i,j}^{(d)} \end{bmatrix}$$

In this equation, the function f is a linear or quadratic function of intake (DMI or MEI), with the parameter vector \emptyset_{ij} and model error e_{ijk} . The experiment and cow(experiment) random effects, $\{b_i\}$ and $\{b_{i,j}\}$, are assumed independent of each other and independent of within cow errors e_{ijk} . The B 's are the fixed effects influencing the curve parameters due to blood flow (PORBF, HEPBF), and are introduced via two dummy variables x_1 and x_2 , respectively.

Initial analysis revealed a potential ‘fan’ shape in the residuals, where residual variance increased with the predicted BF value. In addition, within-treatment and across treatment BF variation increased as BF and/or DMI increased ($P < 0.05$; data not shown). This may reflect the different type of animals used at low and high DMI (beef cattle vs. dairy cows), milk yield or body reserve mobilization, or the range of diets examined. To compensate, a variance weighting statement (**wt**) was added to the NLMM macro model, $wt = 1/(\text{predicted value})^2$, which decreased variance weight with increasing predicted BF value (see Strathe et al., 2009 for discussion).

The joint distribution of random effects was assumed to be multivariate normal and the dual quasi-Newton technique was used for optimization with an adaptive Gaussian quadrature as the integration method.

Equation Evaluation

Goodness of fit of the statistical model (inclusion/exclusion of random effects, variance/covariance structure selection etc.) was evaluated using the Bayesian information criterion (**BIC**) fit statistic (SAS Inst. Inc., Cary, NC), where lower values indicate better model fit, and the value and significance of the fixed effect model parameters were tested against a P value of 0.05.

Evaluation of newly developed and extant equations against the evaluation database were performed via two methods. Firstly, root mean square prediction error (**rMSPE**) was performed, where the mean square prediction error (**MSPE**) is calculated as:

$$MSPE = \sum_{i=1}^n (O_i - P_i)^2 / n \quad [2]$$

where n is the total number of observations, O_i is the observed value, and P_i is the predicted value. The rMSPE, expressed as a percentage of the observed mean, gives an estimate of the overall

prediction error. The rMSPE can also be decomposed into error in central tendency or mean bias (ECT), error due to deviation of the regression slope from unity (ER) and error due to the disturbance (random error) (ED) (Bibby and Toutenburg, 1977).

Secondly, concordance correlation coefficient analysis (CCC) was performed (Lin, 1989), where CCC is calculated as:

$$CCC = r \times C_b \quad [3]$$

where r is the Pearson correlation coefficient and C_b is a bias correction factor. The r variable gives a measure of precision, while C_b is a measure of accuracy. Associated CCC variables (used in calculation of C_b) are v , which provides a measure of scale shift, and u , which provides a measure of location shift relative to the scale. The v value indicates the change in standard deviation, if any, between predicted and observed values. A v value greater than 1.0 indicates larger variance in the predicted data compared to observed, while a v value less than 1.0 indicates a smaller variance in the predicted data compared to observed. A positive u value indicates over-prediction, while a negative u value indicates under-prediction.

RESULTS AND DISCUSSION

Low vs. High Intake

Visual inspection of the data revealed two potential clusters within the databases, representing a cluster of ‘lower-intake’ and ‘higher-intake’ data (Figure 1). These intake groups are confounded with animal type, and also represent clusters of studies, where the low intake group comprised all beef cattle data and the high intake group comprised all dairy cow data. As a result, analysis was initially performed by separating the data (by studies) into low and high intake groups

(or, alternatively, animal type) (Table 1), and analysing for statistical differences between intake-group parameter estimates. In sheep, Vernet et al. (2005; 2009) suggested that BF responses to DMI or MEI differed based on the level of intake. Additionally, physiological status may have an effect on BF. A major difference between the data of Vernet et al. (2005; 2009) and the current data (aside from species) is, however, that in the current database level of intake did not fall far below maintenance requirements (Table 1). In this study, the average multiple of maintenance feeding level was 1.31 (± 0.378) for the low-intake and 2.65 (± 0.749) for the high-intake groups, compared to 0.5 and 1.3 in the study of Vernet et al. (2009), respectively. Separation of studies into two intake groups in the current dataset did not result in significantly different parameter estimates between low- and high-intake groups ($P > 0.09$) (Table 3). As a result, separate equations for the low intake and high intake groups (or animal type) are not reported, and equations reported were fit to the full database.

Linear and Quadratic Blood Flow Equations

Results of linear and quadratic curve fitting to the BF development database are presented in Table 3. Equations were fit to data with BF units of L/h combined with DMI or MEI units of kg/d or MJ/d, or with BF units of L/kg BW^{0.75}/h combined with DMI or MEI units of kg/kg BW^{0.75}/d or MJ/kg BW^{0.75}/d. Scaling relative to BW was also examined, but resulted in no improvements over BW^{0.75}, and is not reported. Model structure (random effects, variance-covariance structures, variance weighting) was optimized to ensure convergence and to minimize the joint BIC value. Joint BIC values represent the BIC for PORBF and HEPBF combined, which were fit simultaneously. The significance of parameter estimates (vs. zero) are reported, as well as the P -value for testing the low vs. high intake parameter estimates against each other, via

CONTRAST statements in SAS (Table 3). This division into low and high intake groups was not performed for quadratic equations due mainly to lack of convergence, but also because a quadratic fit should inherently capture changes in the slope of the relationship across intake level. In support of the findings that parameter estimates did not differ significantly between low and high intake groups, fitting quadratic equations to the database resulted in similar or marginally better joint BIC values, and the quadratic parameter estimates were not always significant (Table 3). Lack of significance of the quadratic parameter indicates potential over-parametrization of the model or that the relationship was linear within the range of data available. When BF was expressed in units of L/h, the negative quadratic parameter was significant for HEPBF, but not for PORBF (driving variable of DMI or MEI). When BF was expressed in units of L/kg BW^{0.75}/h, the quadratic parameter was only significant for PORBF with DMI as a driving variable. Linear equation parameters (slope and intercept) were always significant ($P < 0.01$).

Equations based on MEI generally had lower BIC values compared to equations based on DMI (Table 3), indicating better model fit. Conclusions on BF units cannot be made based on BIC, as BIC values are scaled by the units.

DMI and MEI Based Equation Evaluation

Equations developed were tested on an independent evaluation database (described in Table 2) to compare prediction precision and accuracy. Although the evaluation database may be considered somewhat small relative to the size of the development database, it does represent a complete dataset, where all variables predicted and evaluated were reported in the publications. Results are presented in Table 4 for PORBF, HEPBF, ARTBF (predicted by difference) and PORBF/HEPBF (% ratio of predicted blood flows) for each equation.

Comparing DMI to MEI as the driving variable, DMI typically resulted in slightly better predictions based on rMSPE and CCC results, except for PORBF/HEPBF (Table 4). This could be the result of added variation or error due to MEI determination. However, Han et al. (2002) also suggested that portal BF responded primarily to bulk fill rather than nutrient supply. Reynolds et al. (1991) suggested that in addition to ME consumed, ME density of the diet affected PORBF and HEPBF via effects of forage content on gut fill and subsequent effects on gut mass and the work of digestion, which may also explain the better relationship for splanchnic blood flow and DMI. Vernet et al. (2009) found a similar lack of improvement with MEI over DMI in predicting BF in sheep. Therefore, it is likely that this observation has a physiological basis rather than being error related.

Comparing linear to quadratic equations, predictions were similar but slightly improved with the linear equations (Table 4). As many of the quadratic parameter estimates were not significant, this is not a surprising result.

Comparing L/h and L/kg BW^{0.75}/h as units for BF, CCC results were in general slightly improved when L/h was used and rMSPE results were in general slightly improved when L/kg BW^{0.75}/h was used (Table 4). Scaling with BW^{0.75} reduced the contribution of non-random error sources (ECT, ER) to the rMSPE total, indicating improved predictions compared with scaling without BW^{0.75}. However for CCC, BW^{0.75} scaling reduced the total CCC via a decrease in C_b , despite a slight increase in r . This difference in results is likely due to differences in division of error within rMSPE and CCC calculations (for a discussion see Ellis et al., 2010). Scaling by BW^{0.75} is presumed to extend the range of data the equations may be applicable on, and thus was of interest when combining dairy and beef data, but it may also introduce additional variation due to BW measurement (difficulty getting a precise scale number, variation in gut fill contribution to

BW, etc.). For whichever reason, these results indicate that scaling by $BW^{0.75}$ may not improve predictions of blood flow over units of L/h, as performance between the equations was similar.

Predictions for PORBF and HEPBF, as evaluated by rMSPE and CCC analysis, were typically very good, with CCC values greater than 0.84 and rMSPE values less than 19% (Table 4). The best predictions of PORBF and HEPBF when blood flow was expressed in L/h, were the linear equations with DMI as the driving variable (P1 and H1 equations; rMSPE = 17.5 and 16.6%, CCC = 0.93 and 0.94, respectively). Similarly, when PORBF and HEPBF were expressed relative to $BW^{0.75}$, the linear DMI equations resulted in slightly better predictions (P5 and H5 equations; rMSPE = 15.4 and 14.9%, CCC = 0.87 and 0.90, respectively). However, in general predictions were similar and good across all equations with only minor differences.

Residual analysis was conducted on the seemingly best performing equations (linear, DMI; L/h and L/kg $BW^{0.75}$ /h), and is displayed in Figure 3. Residuals plotted against predicted BF (Figure 3) did not reveal any significant trends in the data ($P > 0.05$), nor for the most part when plotted against the driving variable DMI (kg/d or g/kg $BW^{0.75}$ /d; $P > 0.05$), with the exception of residual ARTBF (L/h), where $P = 0.04$ (residual ARTBF (L/h) = $40.2 (\pm 29.2) - 6.4 (\pm 2.83) \times \text{DMI}(\text{kg/d})$; graphs not shown). The residuals were also plotted against the forage proportion (FP, %) of the diet, and while the regression was not significant for ARTBF, PORBF or HEPBF ($P > 0.05$), it was significant for PORBF/HEPBF (%) ($P = 0.03$ and 0.03 , for L/h and L/kg $BW^{0.75}$ /h equations, respectively; Figure 4). As the result of the FP pattern in the residuals, the FP of the diet was considered as an additional driving variable. The results of separating forage and concentrate DMI is outlined in the following section.

Separating Forage and Concentrate DMI

To further examine the potential effect of the FP of the diet, DMI was separated into forage and (starch-rich) concentrate components (kg/d) in the developmental database, and new equations were parameterized for PORBF and HEPBF, with ARTBF again calculated by difference. Equations developed are presented in Table 5.

When testing the PORBF forage and concentrate slopes against each other, the difference between parameter estimates was non-significant (Table 5), indicating no difference in effect of type of DMI on PORBF. However, testing HEPBF forage and concentrate slope parameters against each other revealed a significant difference, the slope for concentrate being higher (Table 5). This result suggests a higher sensitivity of HEPBF to FP or energy intake compared to PORBF. In support of this, the slope of MEI based equations was also generally higher for HEPBF than for PORBF (Table 3). This may reflect an increased absorption and liver metabolism of propionate and other VFA with an increasing concentrate proportion in diet DM (Huntington, 1990).

Dividing DMI into forage and concentrate components resulted in improved joint BIC values (Table 3 vs. Table 5), slightly improved ARTBF and PORBF/HEPBF predictions, and similar PORBF and HEPBF predictions to equations based on total DMI (Table 4 vs. Table 6).

Interpretation of these FP equations is challenging. For PORBF, it appears forage and concentrate DMI do not differ in their magnitude of effect on BF (similar parameter estimates). This may, however, be the compound result of two opposing mechanisms: forage DMI may stimulate BF less than concentrate DMI due to lower energy content and digestibility, but this may be countered by a higher bulk fill value which is stimulatory to BF (Reynolds et al., 1991).

In contrast, it appears that HEPBF may be more sensitive to concentrate (or energy intake) than to forage intake (significantly different parameter estimates), suggesting that total liver BF is

still more heavily regulated by energy status and absorption of VFA and other components of ME than gut fill. Vernet et al. (2009) made similar observations in sheep.

While these differences did not greatly alter PORBF and HEPBF predictions, prediction of the calculated ARTBF and PORBF/HEPBF were both improved. This suggests that while DMI alone may predict PORBF or HEPBF adequately, differences between them (ARTBF) may be better predicted with consideration of the diet FP. While Vernet et al. (2009) did not examine residuals of arterial/venous BF against FP, they did observe a significant relationship between the residuals and OM digestibility, suggesting again that BF depend on both bulk and the nutrient density of the diet. In order to better understand these effects, an examination of the regulation of liver BF is required.

Blood flow regulation through splanchnic tissues

Blood flow through the portal vein (PORBF), the main blood supply to the liver, is regulated by the portal drained viscera (**PDV**) which is responsible for nutrient uptake and delivery to the post-absorptive environment, as opposed to being controlled by the liver (Lautt, 2009). Bulk fill as well as nutrient delivery to the animal impact this flow (e.g. see Reynolds et al., 1991) through regulation by intrinsic and extrinsic mechanisms. Intrinsic mechanisms include local metabolic control (response to oxygen supply and demand), myogenic control (transmural pressure), local reflexes (presence of lumen contents) and locally produced vasoactive substances (e.g. gastrin, secretin, cholecystokinin) (Lautt, 1996; Lautt, 2009). The extrinsic factors include sympathetic innervation, circulating vasoactive substances and systemic haemodynamic changes (Lautt, 1996; Lautt, 2009). Hepatic arterial blood flow (ARTBF), while regulated by local tissue oxidation levels in other organs, is also not regulated by the liver (Lobley et al., 2000). Instead, it

appears that ARTBF regulation is linked to PORBF, ensuring the liver receives a constant total blood flow relative to liver mass (Lautt, 1996; Lautt, 2009). This appears to be regulated via a continuous release of adenosine into the space of Mall, independent of oxygen supply or demand, followed by removal through both ARTBF and PORBF. Adenosine itself is a powerful vasodilator (Lautt, 2009). If PORBF is reduced, the local concentration of adenosine increases, stimulating arterial vasodilation and increased ARTBF to remove the adenosine. On the other hand, when PORBF is high, e.g. during peak absorption of nutrients from the rumen, this may cause a reduction in ARTBF due to a decrease in local adenosine concentrations. This process is referred to as the hepatic arterial buffer response. In this respect, the liver does not drive either of the incoming blood flows; PORBF is driven by the PDV, and ARTBF is driven, inversely, by PORBF. However, the liver can have significant indirect regulatory effects on incoming BF, via mechanisms impacting BF to splanchnic organs that drain into the PORBF. As well, longer-term effects on BF can be mediated by changes in liver mass. For a full review of liver BF regulation, see Lautt (2009).

Based on the empirical blood flow prediction equations developed in the present work, it is possible that stimulation of PORBF by concentrate (energy) intake is countered by a depression in PORBF by a lower forage intake (bulk fill), resulting in similar forage and concentrate parameters for PORBF prediction across a range of FP. When FP was low and total DMI alone was the driving variable, PORBF/HEPBF was over predicted ($P < 0.05$) and as a result ARTBF slightly under predicted (non-significant; Figure 4). This makes sense as ARTBF is calculated as HEPBF – PORBF. At a low FP, over-prediction of PORBF/HEPBF could be due to over prediction of PORBF and/or under prediction of HEPBF. Examination of the (albeit non-significant) slope terms in Figure 4, suggest that both are occurring to some extent.

Since parameterization with separate forage and concentrate DMI resulted in similar parameters for PORBF, if these results reflect *in vivo* observations, it suggests that for low FP diets, HEPBF is under-represented by using total DMI because of an under-represented ARTBF contribution. This suggests that while total blood flow through the liver is sensitive to energy intake (and thus different forage and concentrate parameters for HEPBF), factors reducing PORBF relative to the local adenosine concentration (in this case, FP or bulk fill) may drive an increase in ARTBF to compensate. Thus, separating forage and concentrate DMI captures this effect of ARTBF, without directly modeling ARTBF.

When interpreting these results, it should be noted that while DMI varied within all studies, FP did not. Although the equations were parameterized on kg/d of forage and concentrate, in the developmental database 5 of 17 studies specifically examined FP effects, and 4 of 9 studies in the evaluation database examined FP effects. The distribution of FP across DMI is illustrated in Figure 2. Therefore, the forage + concentrate equations require examination on an additional database with additional variation in FP to ensure it is not only an artifact of the data used.

Equations Based on Diet Chemical Composition

Although one of the main purposes of this paper was to compare DMI and MEI as the major drivers of PORBF and HEPBF in a multivariate analysis, CP and NDF content of the diet were also available in the development databases. Therefore, initially, development of equations based on CP or NDF intake (kg/d or g/kg BW^{0.75}/d) were also considered. However, while these equations had BIC values comparable to the forage + concentrate DMI equations (joint BIC values were: 6413 for CP (kg/d), 6534 for NDF (kg/d), 1391 for CP (g/kg BW^{0.75}/d), and 1491 for NDF (g/kg BW^{0.75}/d) based equations), their rMSPE and CCC values were worse than those of DMI

and MEI (for e.g., CP (kg/d) predicting PORBF (L/h) resulted in $rMSPE\% = 37.8$, $CCC = 0.63$ and HEPBF (L/h) $rMSPE\% = 37.9$ and $CCC = 0.69$, on the evaluation database). As a result, these equations were not pursued further. However, equations developed considering multiple chemical components of the diet may be considered in the future, in particular given the relationship observed here with FP.

Comparison with Extant Blood Flow Equations

To compare predictions of the newly developed blood flow equations to extant equations, several equations were selected from the literature and applied to the evaluation database. The equations of Lescoat et al. (1996) were not included, as the evaluation database used here shared data with the developmental database used by Lescoat et al. (1996), resulting in unsurprisingly good blood flow predictions by these equations (not shown). Although the equations of Vernet et al. (2009) were developed on sheep, it represented an interesting challenge to include their equations for comparison on cattle data.

Extant equation evaluations are presented in Table 7. Of the equations evaluated, the PORBF equation of Huntington et al. (1984) based on MEI performed comparably to the newly developed PORBF equations in terms of $rMSPE$ and CCC analysis. These equations (Huntington et al., 1984) were developed on beef and dairy heifer data. The linear PORBF equation of Bermingham et al. (2008) performed adequately, with slightly more bias (over prediction) and lower CCC values. However, similar to the results found in the current study, the quadratic equation for PORBF by Bermingham et al. (2008) did not improve predictions over their linear equation. These equations were developed on a combination of sheep and cattle data.

The sheep equations of Vernet et al. (2009) also tended to over predict PORBF, HEPBF and ARTBF, expressed relative to BW, likely illustrating a species difference. Of the 3 sets of extant equations, only those of Vernet et al. (2009) allowed calculation and evaluation of ARTBF and PORBF/HEPBF. Both the Vernet et al. (2009) above maintenance and above + below linear equations tended to under predict the mean PORBF/HEPBF. Interestingly, the Vernet et al. (2009) sheep equations also showed a relationship between the PORBF/HEPBF residual and the FP of the diet (Figure 5) with a trend similar to that in the equations derived in the present study (Figure 4), and therefore seems to support the separation of forage and concentrate parameters.

CONCLUSIONS

Equations developed herein represent advancement over current PORBF, HEPBF, ARTBF and PORBF/HEPBF prediction equations available in the literature for cattle. In the present analysis, a more advanced meta-analysis technique was used, allowing simultaneous predictions of multiple blood flows, as well as providing new equations which separate forage DMI from concentrate DMI, resulting in improvements in ARTBF and PORBF/HEPBF predictions. All PORBF and HEPBF equations performed well when evaluated on an evaluation database. These equations can be applied within a post-absorptive model of cattle metabolism, in order to predict nutrient fluxes to and from the liver, but should be further evaluated on additional data obtained under a wider range of conditions.

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586 **Table 1.** Summary of the blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) developmental database¹.

Variable	<u>All data</u>		<u>Beef cattle (Low-intake group)</u>				<u>Dairy cow (High-intake group)</u>			
	Mean	SD ²	Mean	SD ²	MIN ³	MAX ⁴	Mean	SD ²	MIN ³	MAX ⁴
DMI (kg/d)	11.8	6.58	5.5	1.11	3.0	8.3	17.3	3.84	7.9	25.1
MEI (MJ/d)	126.4	74.91	60.6	12.74	37.4	97.6	192.8	48.03	81.4	295.1
CP (kg/d)	1.8	1.03	0.9	0.25	0.5	1.4	2.6	0.81	1.0	4.6
NDF (kg/d)	3.7	2.44	1.3	0.58	0.6	3.3	5.9	1.13	3.3	9.0
BW (kg)	510	140.3	412	84.4	251	598	637	85.2	487	878
DMI (g/kg BW ^{0.75} /d)	96.3	46.93	61.0	13.94	42.6	104.1	142.2	32.85	57.0	202.6
MEI (MJ/kg BW ^{0.75} /d)	1.01	0.515	0.68	0.175	0.49	1.13	1.55	0.423	0.56	2.26
MEI (Multiple of MN ⁵)	1.81	0.850	1.31	0.378	0.86	2.27	2.65	0.749	0.90	3.90
Forage Proportion (%)	44	18.0	41	23.3	25	75	47	10.3	35	66
ARTBF (L/h)	234	206.4	91	64.2	3	437	359	207.1	18	1089
PORBF (L/h)	1188	586.6	650	126.9	382	986	1655	398.6	762	2887
HEPBF (L/h)	1409	708.7	736	138.3	428	1019	1992	431.0	929	3208
ARTBF (L/kg BW ^{0.75} /h)	1.8	1.52	1.0	0.69	0.0	4.1	2.8	1.66	0.5	8.8
PORBF (L/kg BW ^{0.75} /h)	10.0	3.88	7.2	1.57	4.6	14.4	13.5	3.00	5.6	19.4
HEPBF (L/kg BW ^{0.75} /h)	11.7	4.79	8.2	1.69	5.5	14.7	16.2	3.57	6.9	21.6
PORBF/HEPBF (%)	86	9.3	88	7.7	57	100	85	9.2	59	100
n (data points)	296	-	137	-	-	-	159	-	-	-
n (treatments)	55	-	22	-	-	-	33	-	-	-
n (studies)	17	-	7	-	-	-	10	-	-	-

587 ¹ Mean & SD reported are based on 'n (data points)'.

588 ² Standard deviation.

589 ³ Minimum value in database.

590 ⁴ Maximum value in database.

591 ⁵MN – maintenance energy requirement.

592 **Table 2.** Summary of the blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) evaluation database¹.

Variable	Mean	SD ²	MIN ³	MAX ⁴
DMI (kg/d)	8.4	4.71	4.3	21.8
MEI (MJ/d)	90.4	43.59	51.1	231.5
CP (kg/d)	1.3	0.89	0.7	4.0
BW (kg)	387	97.5	198	538
DMI (g/kg BW ^{0.75} /d)	94.0	36.91	57.1	204.6
MEI (MJ/kg BW ^{0.75} /d)	1.02	0.346	0.62	2.17
MEI (Multiple of MN ⁵)	1.99	0.613	1.16	3.94
Forage Proportion (%)	42	22.0	10	100
ARTBF (L/h)	165	137.9	26	563
PORBF (L/h)	832	369.3	336	1992
HEPBF (L/h)	996	495.9	400	2524
ARTBF (L/kg BW ^{0.75} /h)	1.8	1.24	0.3	5.3
PORBF (L/kg BW ^{0.75} /h)	9.4	2.95	6.4	18.7
HEPBF (L/kg BW ^{0.75} /h)	11.2	3.96	7.5	23.7
PORBF/HEPBF (%)	85	5.9	76	97
n (data points)	34			
n (treatments)	34			
n (studies)	9			

593
594 ¹ Mean & SD reported are based on 'n (data points)'.

595 ² Standard deviation.

596 ³ Minimum value in database.

597 ⁴ Maximum value in database.

598 ⁵MN – maintenance energy requirement.

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600 **Table 3.** Summary of portal (PORBF) and hepatic venous blood flow (HEPBF) prediction equations based on DMI and MEI¹.

Response Variable	Driving Variable	Eqn	ID	Joint BIC	Int	SE	P	P (Intake Level) ²	Slope (Lin)	SE	P	P (Intake Level) ²	Slope (Quad)
<u>L/h</u>	<u>kg/d or MJ/d</u>												
PORBF	DMI	Linear	P1	7303	202	45.6	<0.01	0.98	83.6	3.11	<0.01	0.73	-
HEPBF	DMI		H1		186	45.4	<0.01	0.64	103.8	3.10	<0.01	0.90	-
PORBF	MEI	Linear	P2	6689	294	43.2	<0.01	0.19	6.8	0.26	<0.01	0.67	-
HEPBF	MEI		H2		264	42.8	<0.01	0.09	8.9	0.26	<0.01	0.96	-
PORBF	DMI	Quad	P3	7296	148	70.9	0.04	-	94.9	12.25	<0.01	-	-0.44
HEPBF	DMI		H3		72	69.9	0.31	-	129.3	12.03	<0.01	-	-1.03
PORBF	MEI	Quad	P4	6698	209	68.1	<0.01	-	8.3	1.07	<0.01	-	-0.01
HEPBF	MEI		H4		110	65.8	0.10	-	11.8	1.02	<0.01	-	-0.01
<u>L/kg BW^{0.75}/h</u>	<u>g or MJ/ kg BW^{0.75}/d</u>												
PORBF	DMI	Linear	P5	1548	2.10	0.417	<0.01	0.50	0.080	0.004	<0.01	0.88	-
HEPBF	DMI		H5		1.91	0.421	<0.01	0.17	0.100	0.004	<0.01	0.15	-
PORBF	MEI	Linear	P6	1337	2.80	0.286	<0.01	0.94	6.61	0.256	<0.01	0.81	-
HEPBF	MEI		H6		2.41	0.286	<0.01	0.43	8.71	0.258	<0.01	0.09	-
PORBF	DMI	Quad	P7	1543	0.58	0.728	0.43	-	0.119	0.016	<0.01	-	-0.0002
HEPBF	DMI		H8		0.84	0.769	0.27	-	0.128	0.018	<0.01	-	-0.0002
PORBF	MEI	Quad	P8	1327	1.53	0.690	0.04	-	9.26	1.465	<0.01	-	-1.09
HEPBF	MEI		H8		1.97	0.701	<0.01	-	9.53	1.499	<0.01	-	-0.30

601
602 ¹Abbreviations: Eqn = equation form, ID = equation name, BIC = Bayesian information criterion, Int = intercept.

603 ² Tested whether slope or intercept for data grouped into 'high' intake (dairy cow) differed from data grouped into 'low' intake (beef
604 cattle), via CONTRAST statements in SAS (data not shown).

605 **Table 4.** Summary of blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) predictions on the evaluation
606 database for ARTBF, PORBF and HEPBF locations, where ARTBF = predicted HEPBF – predicted PORBF, PORBF and HEPBF are
607 according to equations presented in Table 3, and PORBF/HEPBF = predicted PORBF/predicted HEPBF \times 100.

Response Variable	Driving Variable	Eqn	ID	Pred Mean ¹	Pred SD ¹	rMSPE, % ²	ECT, % ³	ER, % ⁴	ED, % ⁵	CCC ⁶	r^7	C_b^8	v^9	u^{10}
<u>L/h</u>	<u>kg/d or MJ/d</u>													
ARTBF	DMI	linear		154	93.8	42.4	2.2	14.0	83.8	0.82	0.93	0.88	0.69	-0.09
PORBF			P1	907	388.3	17.5	27.0	9.0	64.1	0.93	0.98	0.95	1.07	0.20
HEPBF			H1	1061	482.1	16.6	15.5	1.1	83.4	0.94	0.99	0.95	0.99	0.13
PORBF/ HEPBF, %				86	1.7	6.6	5.7	0.2	94.1	0.18	0.52	0.34	0.30	0.42
ARTBF	MEI	linear		160	90.2	44.2	0.5	14.8	84.7	0.80	0.92	0.87	0.66	-0.05
PORBF			P2	909	292.0	17.6	27.7	13.8	58.5	0.90	0.95	0.95	0.80	0.24
HEPBF			H2	1068	382.2	18.7	14.8	19.5	65.7	0.91	0.96	0.95	0.78	0.17
PORBF/ HEPBF, %				86	2.4	6.5	3.2	0.3	96.5	0.24	0.68	0.35	0.41	0.27
ARTBF	DMI	quad		160	93.6	44.3	0.5	10.4	89.1	0.80	0.93	0.86	0.69	-0.05
PORBF			P3	908	391.1	17.7	26.7	10.0	63.2	0.93	0.98	0.95	1.07	0.20
HEPBF			H3	1067	484.3	17.3	17.0	1.5	81.4	0.94	0.99	0.95	0.99	0.15
PORBF/ HEPBF, %				86	2.2	6.9	2.8	3.3	93.9	0.13	0.65	0.20	0.38	0.27
ARTBF	MEI	quad		166	90.8	45.4	0.0	11.6	88.3	0.79	0.92	0.86	0.67	0.01
PORBF			P4	910	299.5	17.4	28.9	10.2	60.9	0.91	0.96	0.95	0.82	0.24
HEPBF			H4	1076	390.0	19.0	17.6	14.8	67.6	0.91	0.96	0.95	0.80	0.18
PORBF/ HEPBF, %				85	2.8	6.7	0.9	4.2	95.0	0.22	0.78	0.28	0.48	0.13
<u>L/kg BW^{0.75}/h</u>	<u>g or MJ/ kg BW^{0.75}/d</u>													
ARTBF	DMI	linear		1.7	0.73	43.3	2.0	10.2	87.8	0.70	0.87	0.80	0.60	-0.12
PORBF			P5	9.6	2.91	15.4	1.6	6.2	92.2	0.87	1.00	0.88	1.00	0.06
HEPBF			H5	11.3	3.64	14.9	0.2	0.5	99.3	0.90	1.00	0.90	0.93	0.02
PORBF/ HEPBF, %				86	1.3	6.6	1.2	0.9	97.9	0.14	0.43	0.32	0.23	0.22

ARTBF	MEI	linear		1.8	0.72	45.0	0.3	7.5	92.2	0.67	0.87	0.77	0.59	-0.05
PORBF			P6	9.6	2.25	14.4	0.7	6.2	93.1	0.86	0.97	0.89	0.78	0.05
HEPBF			H6	11.3	2.97	15.6	0.2	10.3	89.6	0.87	0.96	0.91	0.76	0.02
PORBF/ HEPBF, %				85	1.8	6.6	0.0	0.0	99.9	0.16	0.56	0.29	0.31	0.02
ARTBF	DMI	quad		1.7	0.80	41.5	3.2	6.7	90.0	0.74	0.91	0.81	0.65	-0.14
PORBF			P7	9.8	2.62	15.9	5.7	0.5	93.8	0.85	0.99	0.87	0.90	0.13
HEPBF			H7	11.5	3.41	15.5	1.6	0.2	98.1	0.89	0.99	0.90	0.87	0.06
PORBF/ HEPBF, %				86	1.9	6.4	4.6	1.5	93.9	0.24	0.56	0.43	0.32	0.35
ARTBF	MEI	quad		1.6	0.84	43.7	4.2	2.0	93.8	0.72	0.92	0.78	0.69	-0.16
PORBF			P8	9.7	2.14	15.4	3.7	8.4	87.9	0.84	0.95	0.88	0.74	0.11
HEPBF			H8	11.4	2.96	15.7	0.4	10.0	89.6	0.87	0.96	0.90	0.76	0.03
PORBF/ HEPBF, %				86	2.4	6.6	5.6	0.4	94.0	0.23	0.67	0.35	0.41	0.36

¹ Where: observed means \pm SD: ARTBF, PORBF, HEPBF (L/h): 165 ± 137.9 , 832 ± 369.3 , 996 ± 495.9 ; ARTBF, PORBF, HEPBF (L/kg BW^{0.75}/h): 1.8 ± 1.24 , 9.4 ± 2.95 , 11.2 ± 3.96 ; PORBF/HEPBF (%): 85 ± 5.9 , respectively.

²Root mean square prediction error, % of observed mean.

³Error due to mean bias, as a % of total MSPE.

⁴Error due to regression, as a % of total MSPE.

⁵Error due to disturbance, as a % of total MSPE.

⁶Condordance correlation coefficient, where $CCC = r \times C_b$.

⁷Pearson correlation coefficient.

⁸Bias correction factor.

⁹Scale shift.

¹⁰Location shift relative to the scale.

621 **Table 5.** Summary of portal (PORBF) and hepatic venous (HEPBF) blood flow prediction equations based on DMI divided into
622 forage (F) and concentrate (C) intake¹.

Response Variable	Driving Variable	Eqn	ID	Joint BIC	Int	SE	<i>P</i>	Slope (F)	SE	<i>P</i>	Slope (C)	SE	<i>P</i>	<i>P</i> (F vs. C) ²
<u>L/h</u>	<u>kg/d</u>													
PORBF	DMI ³	Linear	P9	6512	210	51.0	<0.01	82.9	6.43	<0.01	82.9	6.04	<0.01	1.00
HEPBF			H9		184	50.6	<0.01	92.6	6.28	<0.01	114.2	5.88	<0.01	0.03
<u>L/kg BW^{0.75}/h</u>	<u>g/kg BW^{0.75}/d</u>													
PORBF	DMI ³	Linear	P10	1365	2.16	0.467	<0.01	0.08	0.006	<0.01	0.08	0.006	<0.01	0.41
HEPBF			H10		1.91	0.468	<0.01	0.09	0.006	<0.01	0.11	0.006	<0.01	0.01

623
624 ¹Abbreviations: Eqn = equation form, ID = equation name, BIC = Bayesian information criterion, Int = intercept.

625 ² Tested whether the forage (F) and concentrate (C) slopes differed from each other, performed via CONTRAST statements in SAS.

626 ³Separated into forage DMI (kg/d) + concentrate DMI (kg/d).

627 **Table 6.** Summary of blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) predictions based on
628 separated forage + concentrate DMI, on the evaluation database for ARTBF, PORBF and HEPBF locations, where ARTBF =
629 predicted HEPBF – predicted PORBF, PORBF and HEPBF predictions are according to equations presented in Table 5, and
630 PORBF/HEPBF = predicted PORBF/predicted HEPBF $\times 100$.

Response Variable	Driving Variable	Eqn	ID	Pred Mean ¹	Pred SD ¹	rMSPE, % ²	ECT, % ³	ER, % ⁴	ED, % ⁵	CCC ⁶	r^7	C_b^8	v^9	u^{10}
<u>L/h</u>	<u>kg/d</u>													
ARTBF	DMI ¹¹	linear	-	160	105.1	41.3	0.6	3.9	95.5	0.84	0.97	0.87	0.77	-0.04
PORBF			P9	909	385.0	17.5	28.4	7.6	63.9	0.93	0.98	0.95	1.06	0.21
HEPBF			H9	1069	485.0	17.6	17.0	1.7	81.3	0.94	0.99	0.95	0.99	0.15
PORBF/ HEPBF %				86	3.3	6.2	4.1	1.0	94.9	0.40	0.84	0.48	0.57	0.24
<u>L/kg BW^{0.75}/h</u>	<u>g or MJ/ kg BW^{0.75}/d</u>													
ARTBF	DMI ¹¹	linear	-	1.7	0.80	42.1	1.1	5.3	93.6	0.73	0.91	0.80	0.66	-0.08
PORBF			P10	9.7	2.91	15.7	3.2	6.2	90.6	0.87	1.00	0.87	1.00	0.09
HEPBF			H10	11.4	3.64	15.5	1.2	0.6	98.2	0.89	1.00	0.90	0.93	0.05
PORBF/ HEPBF %				85	2.9	6.2	0.8	0.5	98.7	0.36	0.80	0.44	0.51	0.11

631
632 ¹ Where: observed means \pm SD: ARTBF, PORBF, HEPBF (L/h): 165 ± 137.9 , 832 ± 369.3 , 996 ± 495.9 ; ARTBF, PORBF, HEPBF
633 (L/kg BW^{0.75}/h): 1.8 ± 1.24 , 9.4 ± 2.95 , 11.2 ± 3.96 ; PORBF/HEPBF (%): 85 ± 5.9 , respectively.

634 ²Root mean square prediction error, % of observed mean.

635 ³Error due to mean bias, as a % of total MSPE.

636 ⁴Error due to regression, as a % of total MSPE.

637 ⁵Error due to disturbance, as a % of total MSPE.

638 ⁶Condordance correlation coefficient, where $CCC = r \times C_b$.

639 ⁷Pearson correlation coefficient.

640 ⁸Bias correction factor.

641 ⁹Scale shift.

642 ¹⁰Location shift relative to the scale.

643 ¹¹Separated into forage DMI (kg/d) + concentrate DMI (kg/d).

644 **Table 7.** Blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) predictions by extant equations on
645 the evaluation database for ARTBF, PORBF and HEPBF locations.

Source	Response Variable	Driving Variable	Eqn	Pred Mean ¹	Pred SD ¹	rMSPE, % ²	ECT, % ³	ER, % ⁴	ED, % ⁵	CCC ⁶	r^7	C_b^8	v^9	u^{10}
Vernet et al. (2009) (above MN)	<u>L/kg BW/h</u>	<u>g/kg BW/d</u>												
	ARTBF	DMI	Lin	0.6	0.17	65.1	57.4	1.0	41.6	0.48	0.64	0.75	0.65	0.96
	PORBF	DMI	Lin	2.6	0.45	26.6	62.2	1.4	36.4	0.59	0.71	0.84	0.73	0.84
	HEPBF	DMI	Lin	3.2	0.62	29.9	72.4	1.5	26.1	0.61	0.69	0.88	0.76	0.91
	PORBF/ HEPBF	DMI	Lin ¹¹	81	1.2	7.7	29.6	1.9	68.5	0.11	0.30	0.37	0.22	-1.32
Vernet et al. (2009) (above + below MN)	<u>L/kg BW/h</u>	<u>g/kg BW/d</u>												
	ARTBF	DMI	Quad	0.6	0.15	66.5	60.1	3.5	36.4	0.45	0.58	0.77	0.58	1.07
	PORBF	DMI	Quad	2.7	0.64	32.4	70.6	3.1	26.3	0.58	0.70	0.82	1.02	0.92
	HEPBF	DMI	Quad	3.3	0.78	34.8	79.8	0.7	19.6	0.59	0.67	0.88	0.96	0.99
	PORBF/ HEPBF	DMI	Lin ¹¹	81	0.1	7.7	22.9	9.1	68.0	0.01	0.03	0.36	0.02	-4.03
Bermingham et al. (2008)	<u>L/kg BW/h</u>	<u>g/kg BW/d</u>												
	PORBF	DMI	Lin	2.4	0.51	20.5	38.6	0.0	61.4	0.74	0.88	0.84	0.82	0.48
	PORBF	DMI	Quad	2.7	1.21	45.4	35.5	51.2	13.4	0.57	0.69	0.82	1.94	0.67
Huntington (1984)	PORBF, L/h	MEI, MJ/d	Lin	876	249.1	18.6	8.3	39.6	52.1	0.88	0.92	0.95	0.68	0.15

646
647 ¹ Where: observed means \pm SD: ARTBF, PORBF, HEPBF (L/h): 165 ± 137.9 , 832 ± 369.3 , 996 ± 495.9 ; ARTBF, PORBF, HEPBF
648 (L/kg BW/h): 0.4 ± 0.26 , 2.1 ± 0.63 , 2.5 ± 0.83 ; ARTBF, PORBF, HEPBF (L/kg BW^{0.75}/h): 1.8 ± 1.24 , 9.4 ± 2.95 , 11.2 ± 3.96 ;
649 PORBF/HEPBF (%): 85 ± 5.9 , respectively.

650 ²Root mean square prediction error, % of observed mean.

651 ³Error due to mean bias, as a % of total MSPE.

652 ⁴Error due to regression, as a % of total MSPE.

653 ⁵Error due to disturbance, as a % of total MSPE.

654 ⁶Condordance correlation coefficient, where $CCC = r \times C_b$.

655 ⁷Pearson correlation coefficient.

656 ⁸Bias correction factor.

657 ⁹Scale shift.

658 ¹⁰Location shift relative to the scale.

659 ¹¹ $\text{PORBF/HEPBF \%} = (100 - \text{Arterial/venous \% linear prediction equation from Vernet et al. (2009)}).$

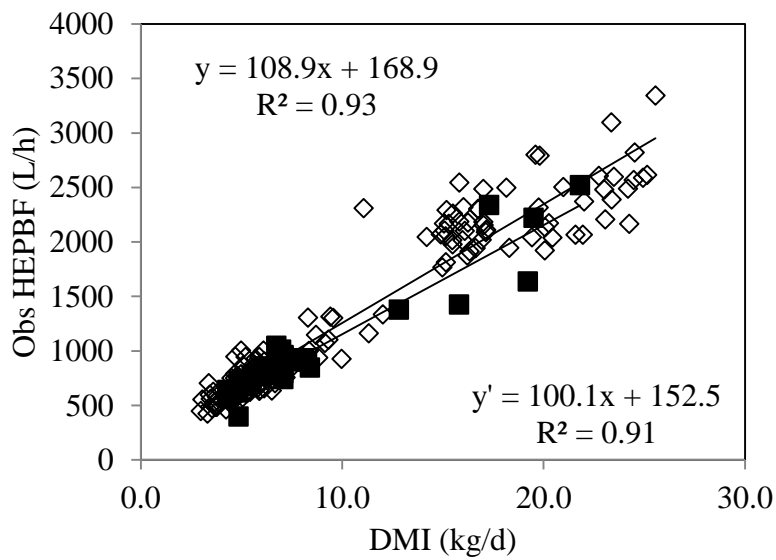
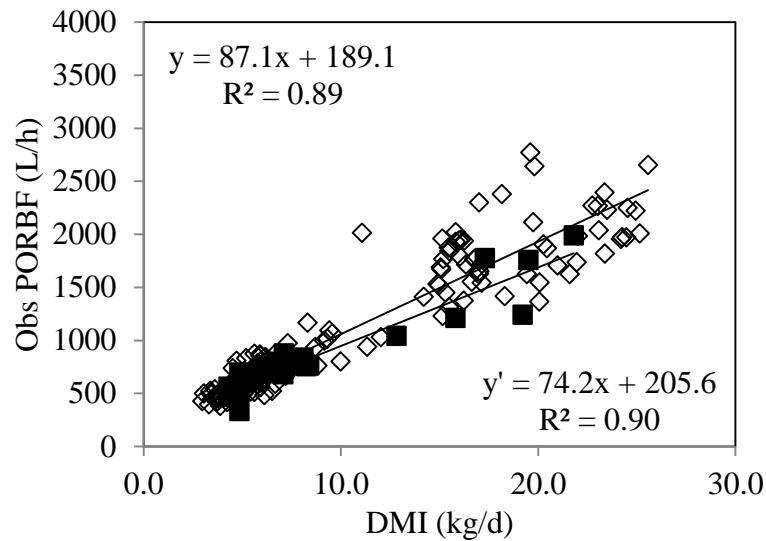


Figure 1. Observed portal blood flow (PORBF; top) and hepatic blood flow (HEPBF; bottom) vs. DMI (kg/d) for the developmental database (\diamond , y) and the evaluation database (\blacksquare , y').

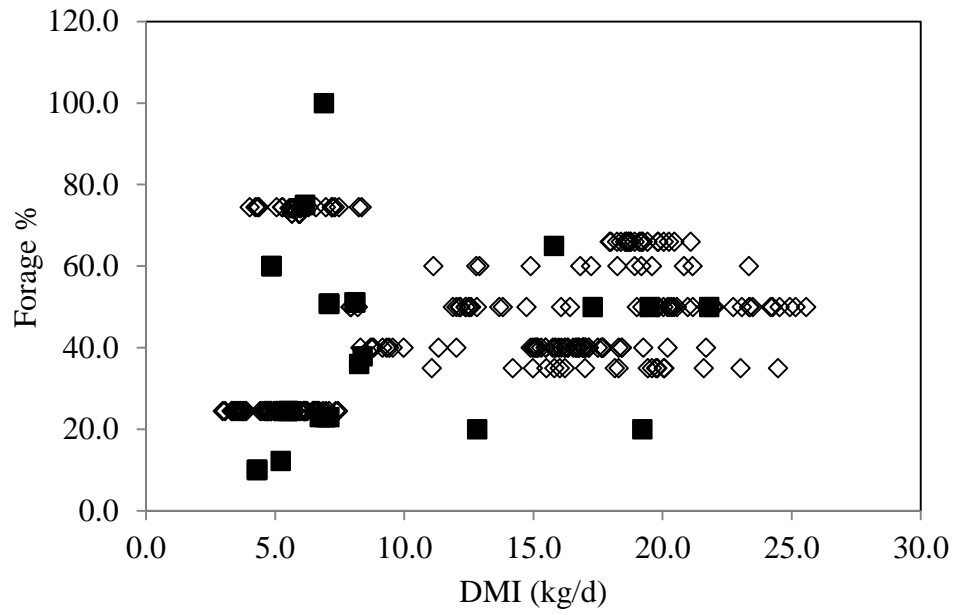


Figure 2. Distribution of forage % across DMI (kg/d) for the developmental (\diamond) and evaluation (\blacksquare) databases.

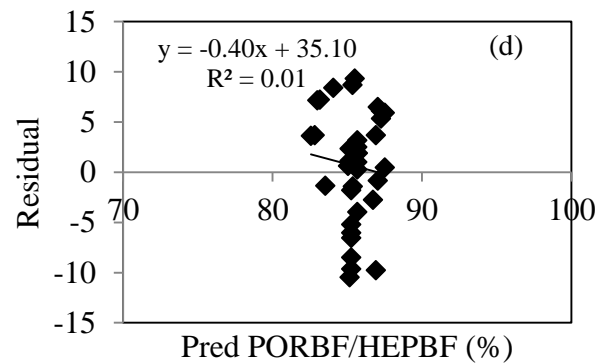
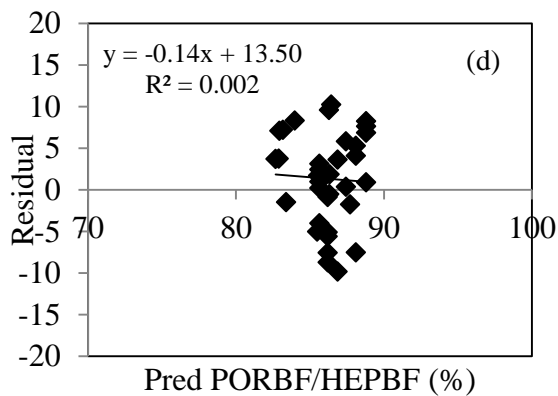
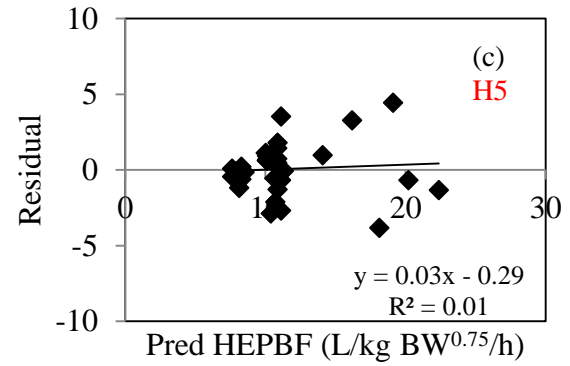
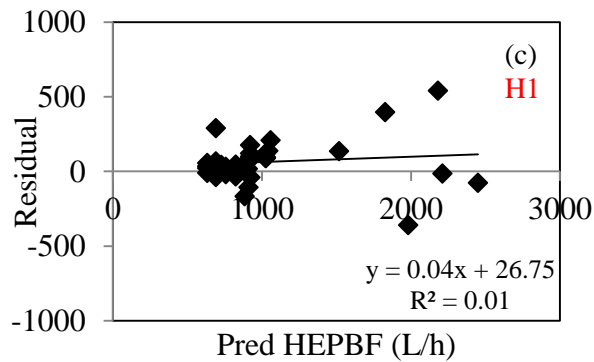
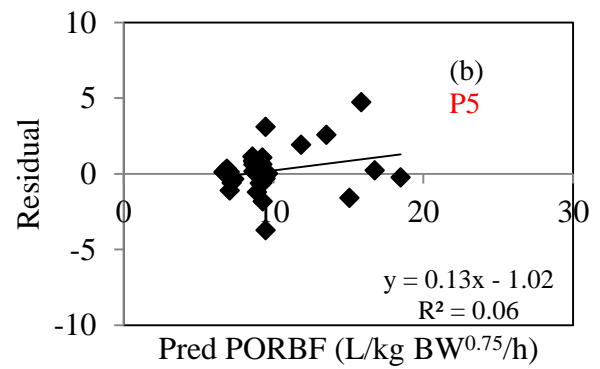
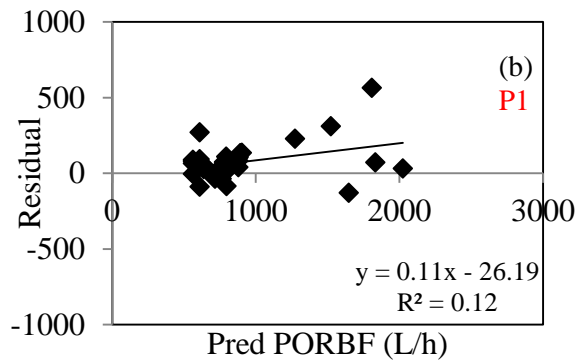
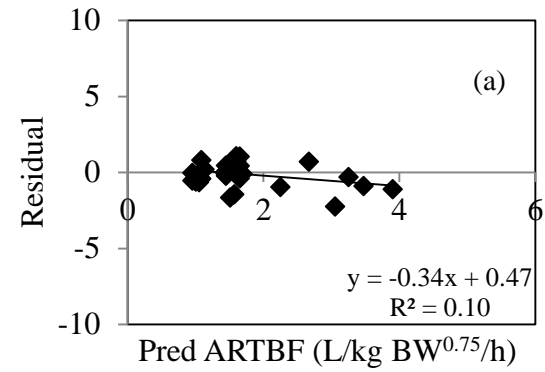
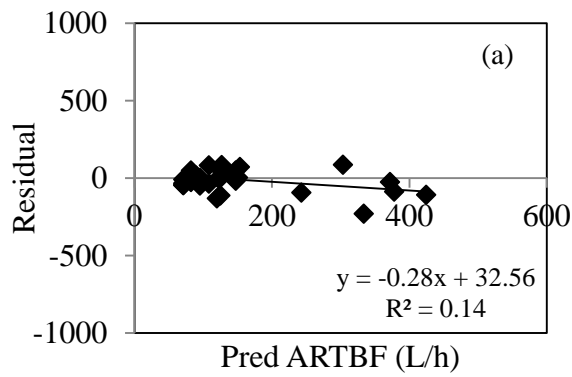


Figure 3. Residual (predicted – observed value) vs. predicted blood flow values for the linear DMI based equations (Table 3) based on blood flow in L/h (left) or L/kg BW^{0.75}/h (right), evaluated on the evaluation database for ARTBF (a), PORBF (b) HEPBF (c) and PORBF/HEPBF % (d), and where ARTBF - hepatic arterial, PORBF - portal venous and HEPBF - hepatic venous blood flows.

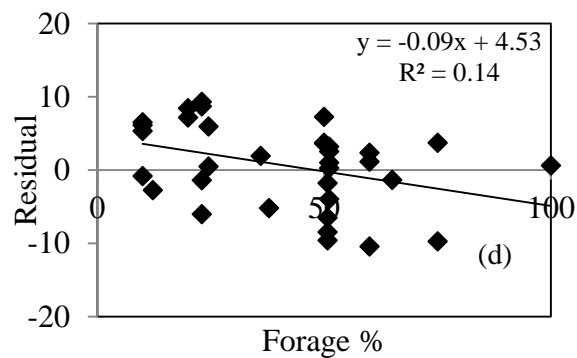
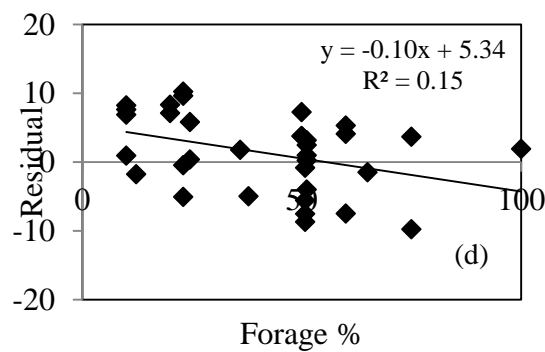
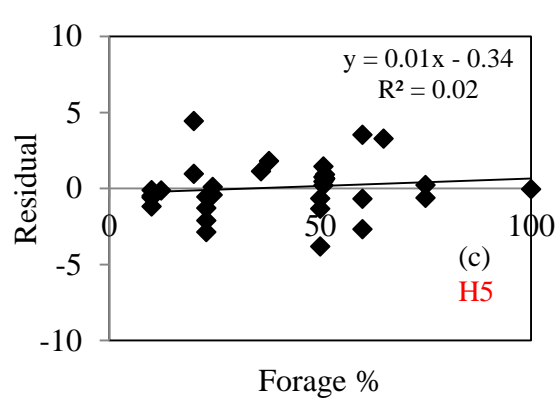
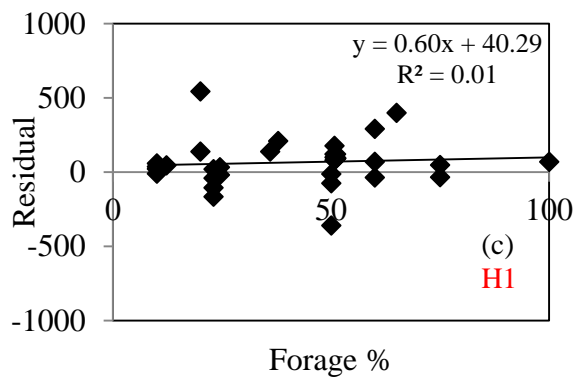
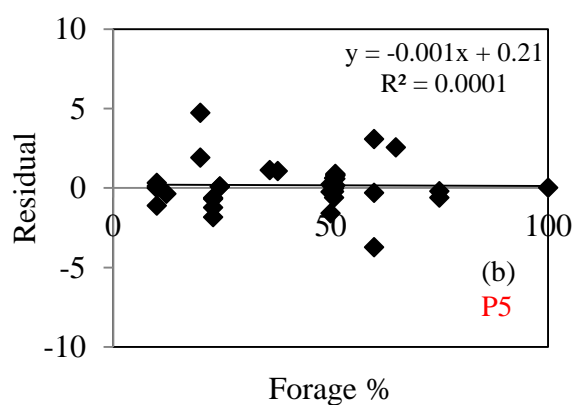
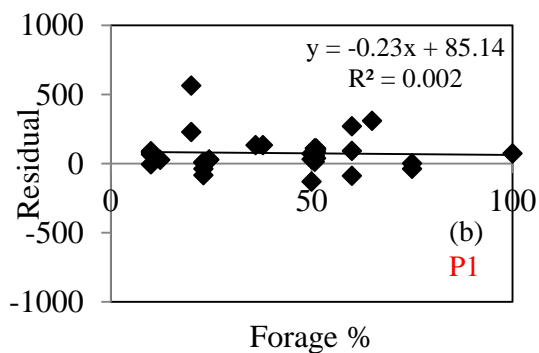
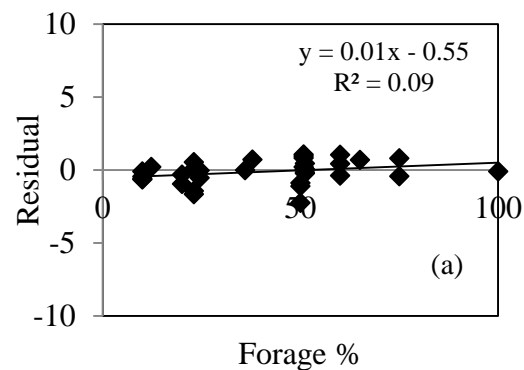
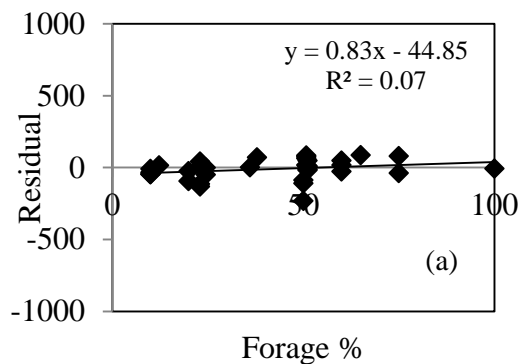


Figure 4. Residual (predicted – observed value) vs. the forage proportion (%) of the diet for the DMI based equations (Table 3) based on blood flow in L/h (left) or L/kg BW^{0.75}/h (right), evaluated on the evaluation database for ARTBF (a), PORBF (b) HEPBF (c) and PORBF/HEPBF (d), and where ARTBF - hepatic arterial, PORBF - portal venous and HEPBF - hepatic venous blood flows.

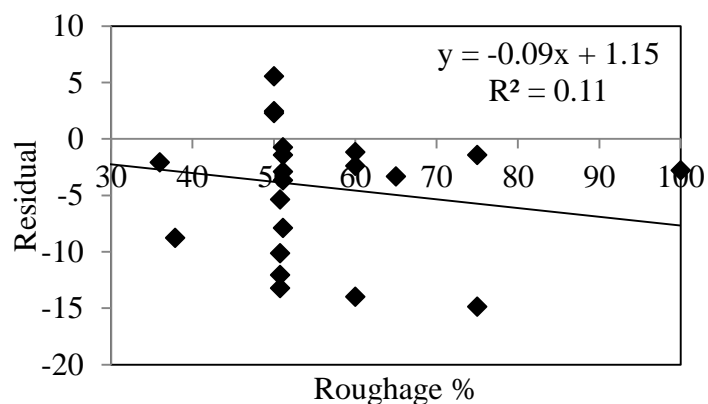
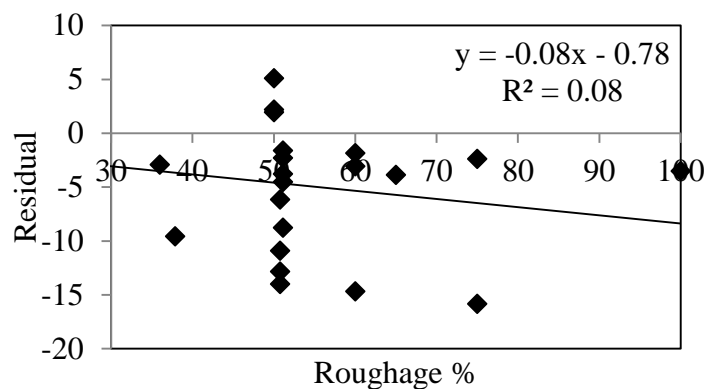


Figure 5. Residual (predicted – observed value) PORBF/HEPBF (%) vs. the forage proportion (%) in the diet for the DMI based sheep equations of Vernet et al. (2009), for their above maintenance equation (linear) (Top), and above plus below maintenance equation (quadratic) (Bottom), evaluated on the evaluation database.